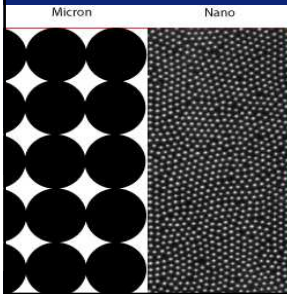


Het toegevoegde risico van grootte



Het kwantificeren van opname, interne localisatie en verdeling van metaalhoudende nanodeeltjes in waterorganismen

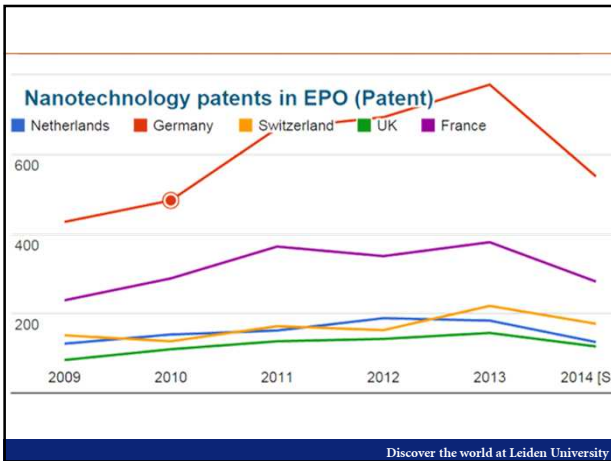
Martina G. Vijver

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Nanodeeltjes rukken op

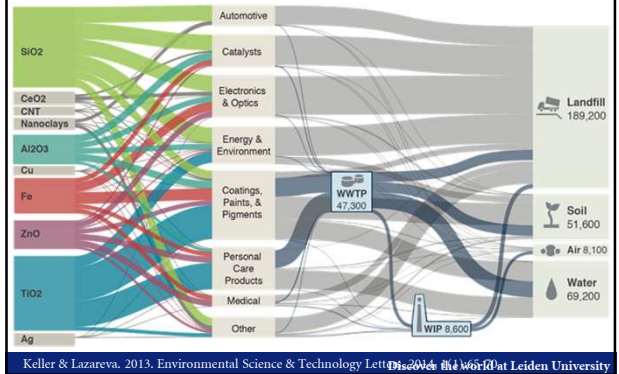


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Globale massa flow ENMs (in metric tons per year) van productie tot afval



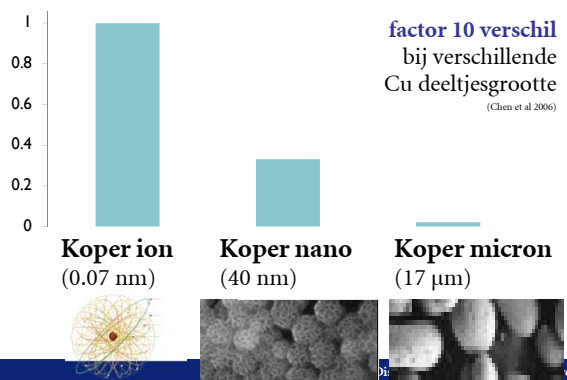
Keller & Lazareva. 2013. Environmental Science & Technology Letters. Discover the world at Leiden University

Definitie nanodeeltje

- Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm (EU Commission)

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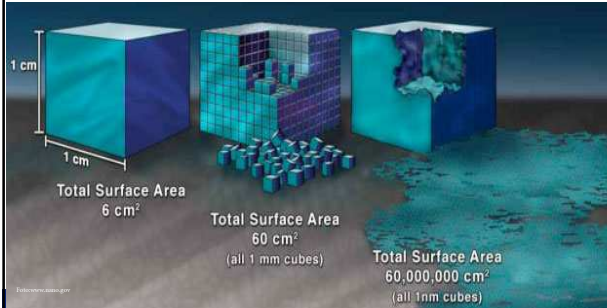
De effecten: sterfte muizen



iversity

Kleine deeltjes hebben ander gedrag dan grotere

Surface area increase while total volume remains constant

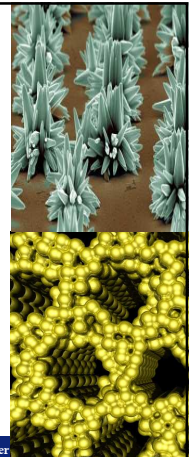


Nanodeeltjes hebben vergrootte reactiviteit

Vanwege nanometer schaal:

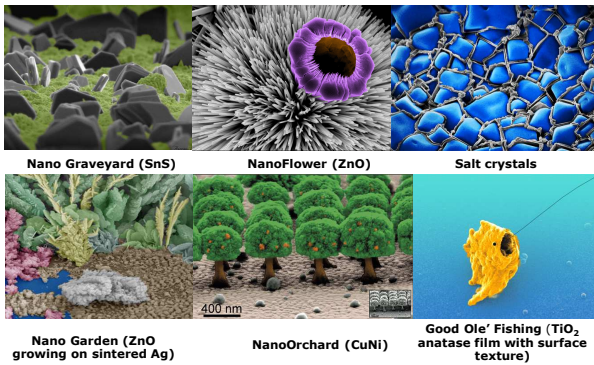
- (i) Veel atomen aan oppervlak;
- (ii) Hoge reactiviteit;
- (iii) Atomen ruimtelijk ingeperkt;
- (iv) Heel weinig onvolkomenheden.

In verhouding tot vergelijkbare bulk-materialen



Discover

Nieuwe vormen van materialen



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Om een uitspraak te kunnen doen over risico's, is meer inzicht nodig in de relatie tussen deeltjesgrootte van materialen en (milieu)gezondheidseffecten (RIVM-website)

Huidige risico-inschatting niet adequaat voor nanodeeltjes

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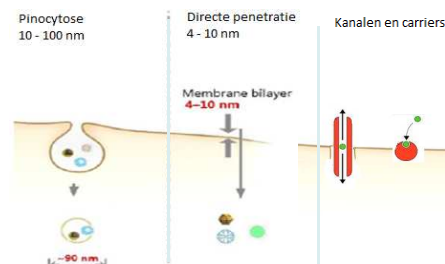
Nanotoxicologie = chemische + deeltjes toxicologie

- Dosimetrie:
 - Chemische compositie
 - Fysische compositie

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Opnametheorie

- Metaal ionen: diffusie door kanalen (Campbell et al. 2005)
- Nanodeeltjes: penetratie, pinocytose (Zhu et al. 2013)
- Microdeeltjes: potentieel via fagocytose



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Identificatie van ecotoxicologische mechanismen

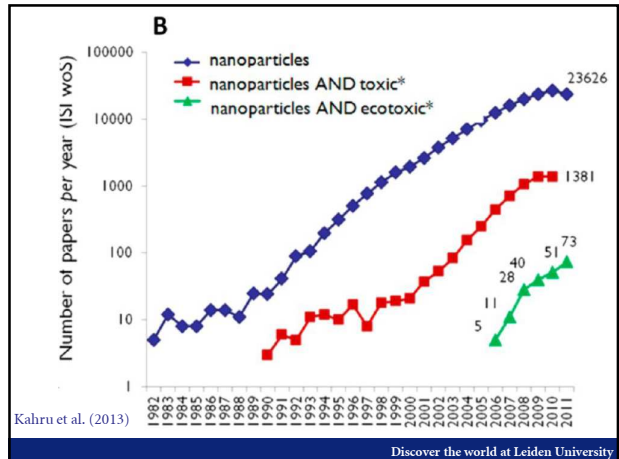
RQ =

Wat is het toegevoegde effect van **grootte** op **opname** en **toxiciteit**?

- metaalhoudende nano-, micron deeltjes, ionen
- voor verschillende aquatische organismen

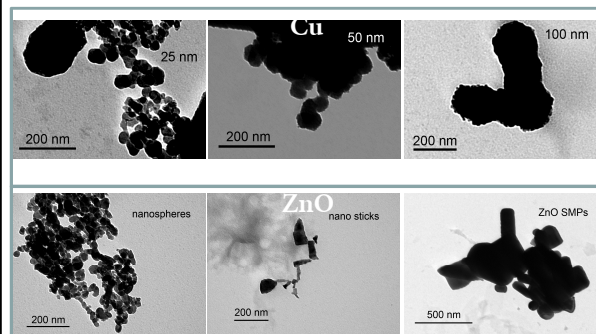
Micro en nano plastics

vorm

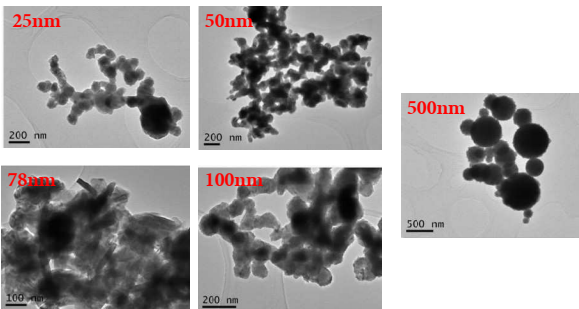


FYSISCH – CHEMISCH GEDRAG

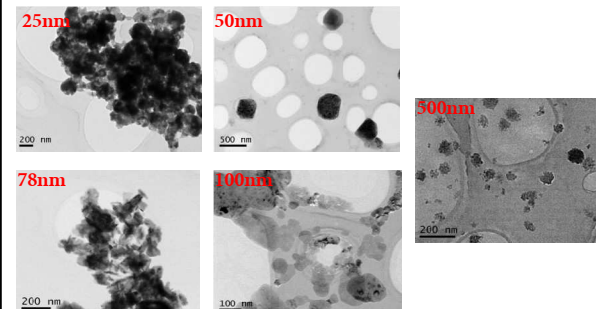
The primary sizes, shapes of NPs

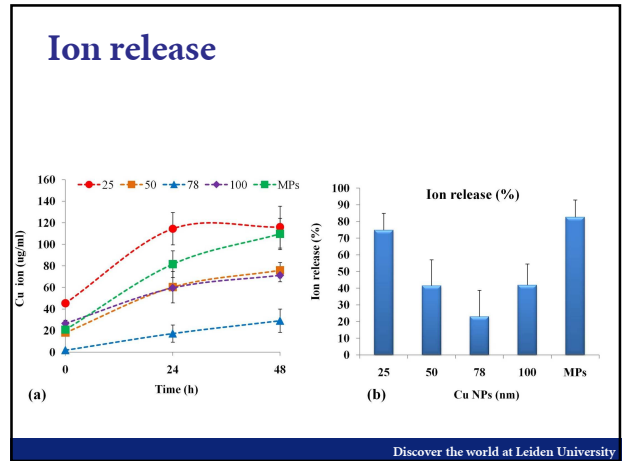
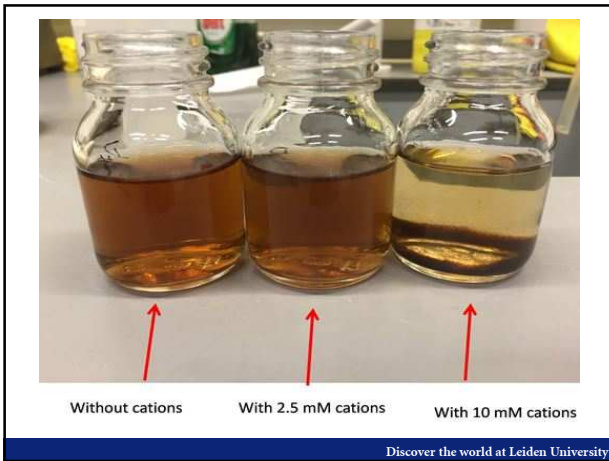


Size of Cu NPs In ethanol



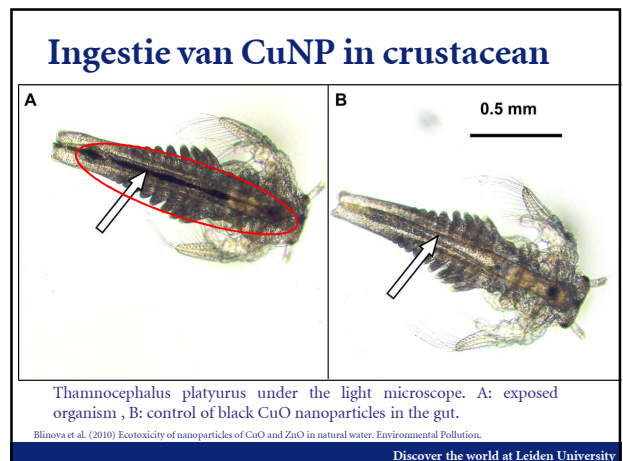
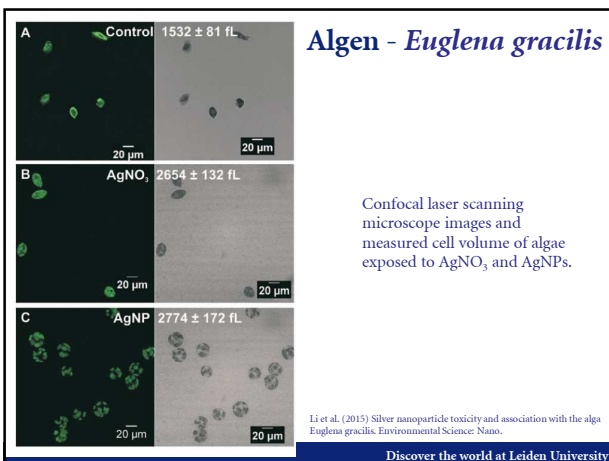
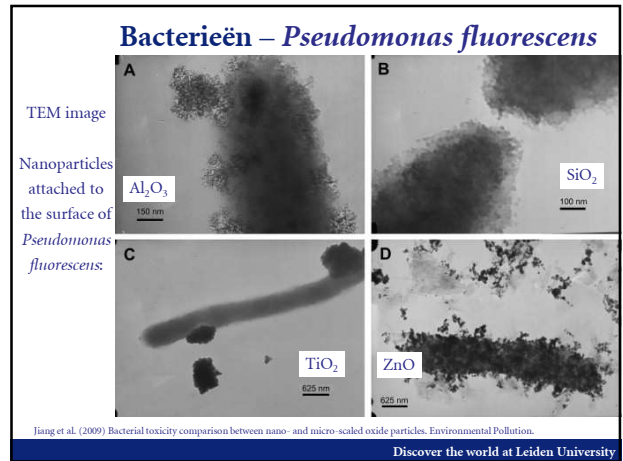
Size of Cu NPs In H4IIE medium after 24 hours





Interacties met organismen

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Distributie van NP in crustacean

Ma et al. (2013)

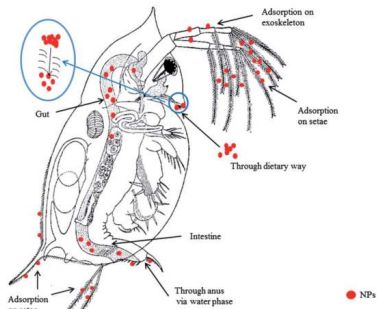


Fig. 3 Uptake routes and distributions of NPs in daphnia. The figure shows both the outer nano-bio interface and inner distribution of NPs. The NPs could be adsorbed on the exoskeleton and setae. The NPs can enter the daphnia through dietary routes or through the anus via water phase – they then mainly accumulate in the digestive system. The NP aggregates could be dispersed by the action of cilia on the gills when entering the organism.

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Fig. 2 *Daphnia magna* ingest nanoparticles from aqueous suspension as indicated by the presence of Cu_2O (A) and TiO_2 (B) in the digestive track (arrows). (A) *D. magna* after 48 h exposure to an aqueous suspension of $3 \text{ mg l}^{-1} \text{ Cu}_2\text{O}$ (99.9%, Sigma-Aldrich) prepared by prolonged stirring. (B) *D. magna* after 48 h exposure to an aqueous suspension of $40.5 \text{ mg l}^{-1} \text{ TiO}_2$ (P25, Degussa) prepared by sonication

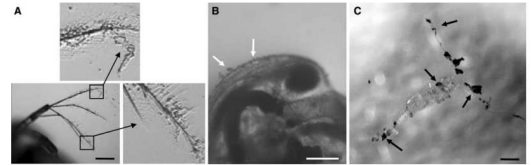
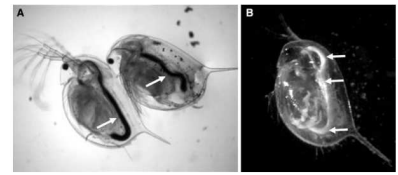
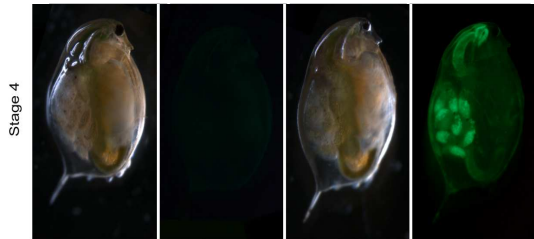


Fig. 3 Adhesion of nanoparticles to the exoskeleton and antennae of *D. magna* and *A. tonsa* was the synthetic M7—salted up to 2% for *D. magna* and *A. tonsa* (Kusk and Wollenberger 1999). Scale bars correspond to 200 μm (A, B) and the marine copepod *Acartia tonsa* exposed to $0.5 \text{ mg l}^{-1} \text{ Cu}_2\text{O}$ (C). Suspensions were prepared as described in Fig. 2. Media for Baun et al. (2008) Ecotoxicity of engineered nanoparticles to aquatic invertebrates: ... recommendations for future toxicity testing.

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NPs in embryo Brun, ... Vijver, in prep

Control Transmission Fluorescence PNP Transmission Fluorescence



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Distributie van NP in vis

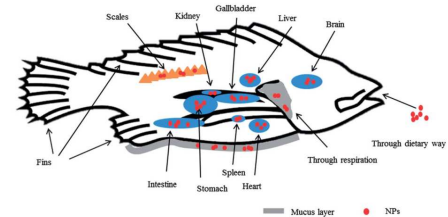


Fig. 4 Adsorption of NPs on the outer surfaces and distribution in the main internal organs of fish. The involved outer interfaces contain scales, mucus layers, fins, and skin. NPs inside the fish may group in some of the organs, including gill, stomach, intestine, gallbladder, liver, kidney, spleen, heart, and brain.

Ma et al. (2013) The biophysicochemical interactions at the interfaces between nanoparticles and aquatic organisms: adsorption and internalization. Environmental Science: Processes & Impacts.

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Accumulation of 10 nm AgNP in adult zebrafish

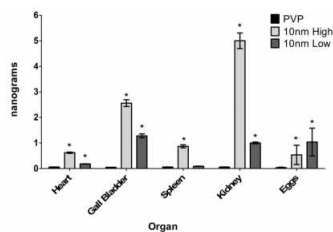


Figure 1. Silver concentration in the organs. Organs or eggs ($n=3$) were assayed for their silver content using ICP-MS following high (5 mg/kg) or low (1 mg/kg) Ag-NP exposure. * denotes significant increase over the PVP control ($p < 0.05$).

Speshock et al. (2016) Differential organ toxicity in the adult zebra fish following exposure to acute sub-lethal doses of 10 nm silver nanoparticles. Frontiers in Nanoscience and Nanotechnology.

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Distributie van NP in zebra vis

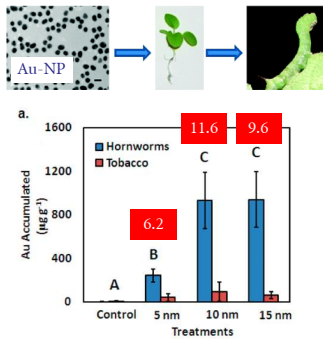


Van Pomeran ... Vijver In prep.

Fluorescence labeled NPs in zebrafish, Cell Observatory Leiden University

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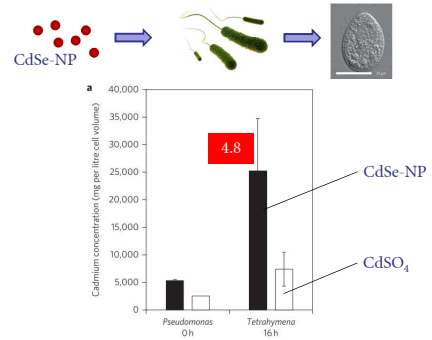
Food-chain transfer



Judy et al. (2011) Evidence for Biomagnification of Gold Nanoparticles within a Terrestrial Food Chain. ES&T.

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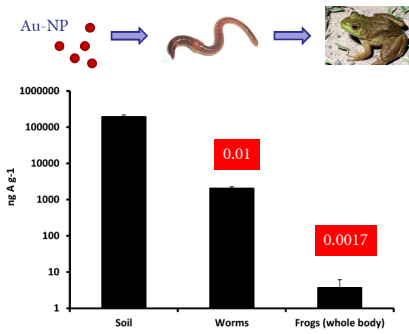
Food-chain transfer



Werlin et al. (2011) Biomagnification of cadmium selenide quantum dots in a simple experimental microbial food chain. Nature Nano.

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Food-chain transfer



Urrine et al. (2012) Trophic transfer of Au nanoparticles from soil along a simulated terrestrial food chain. ES&T.

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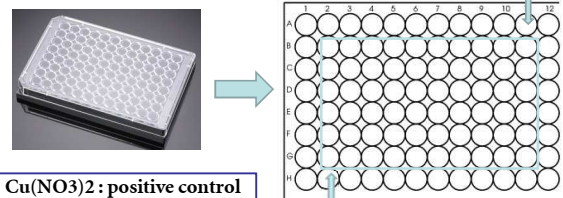
BIOLOGISCHE RESPONSE

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Cellijnen

	HepG-2	H4IIE	PLHC-1	RTH-149
Human	Hep-G2	H4IIE	PLHC	RTH 149
	37 °C, 5% CO ₂	37 °C, 5% CO ₂	30 °C, 5% CO ₂	20 °C, 5% CO ₂
	500 ml EMEM Eagle Medium add: 50 ml fetal bovine serum, 5 ml penicillin-streptomycin, 5 ml Ultr glutamine, 5 ml NEAA	500 ml EMEM Eagle Medium add: 50 ml fetal bovine serum, 5 ml penicillin-streptomycin, 5 ml Glutamine, 5 ml NEAA	500 ml MEM Medium add: 25 ml fetal bovine serum, 5 ml penicillin-streptomycin, 5 ml Glutamine	500 ml EMEM Eagle Medium add: 50 ml fetal bovine serum, 5 ml penicillin-streptomycin, 5 ml Glutamine, 5 ml NEAA
	75,000	25,000	50,000	25,000

Exposure



Five Cu NPs

25nm, 50nm, 78nm, 100nm and 500nm
Highest exposure concentration: 200mg/l

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Doel

Cytotoxiciteit van verschillende Cu NPs in verschillende hepatoma cellijnen

The preliminary hypotheses

1. Toxicity of Cu NPs could be related with the type of cells.
2. Toxicity of Cu NPs increase with the decrease of particle size.

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Resazurin assay (blue dye) Viability of cells

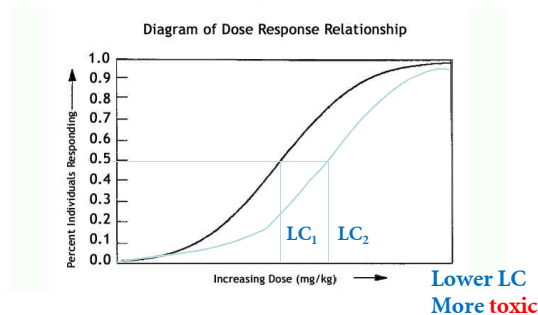


Response Additie

$$E(c_{\text{mix}}) = 1 - \prod_{i=1}^n (1 - E(c_i))$$

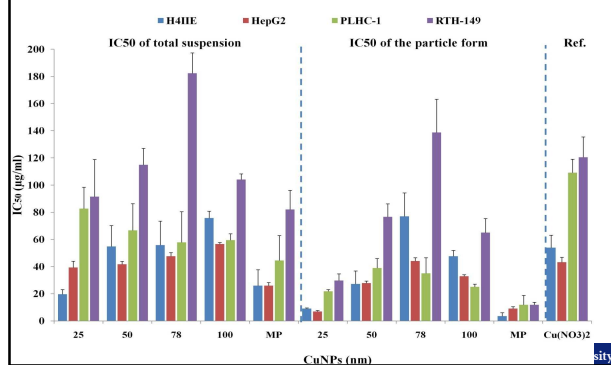
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Dose response



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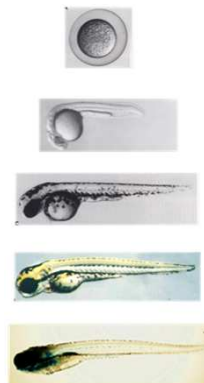
IC50 of nano copper in each cell lines



Results

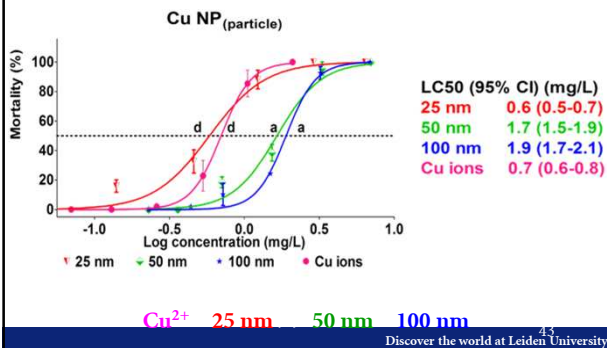
- Mammalian cell lines are more sensitive to Cu NPs than fish cell lines
- The Needle shape of Cu NPs (78nm) is more stable in medium and less toxic compare to round shape of Cu NPs (25nm, 50nm, 100nm and 500nm)
- Ion release of Cu NPs could significantly contribute to the toxicity of Cu NPs.
- No clear relation between size of NPs and toxicity effect

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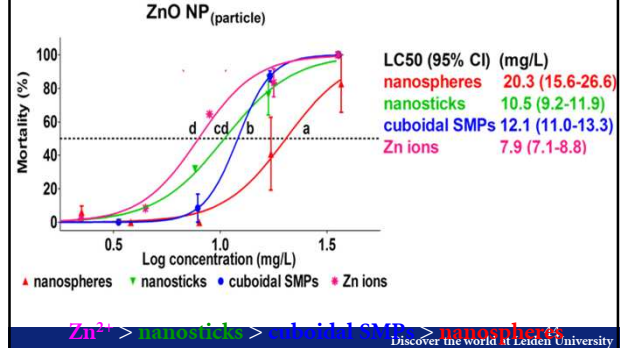


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LC50s values of Cu NPs



LC50s values of ZnO NPs



Relative contribution

Cu NPs	LC50 of NP _(total) (mg/L)	Toxic contribution (%)	
		NP _(ion)	NP _(particle)
25 nm	1.0	0.4	99
50 nm	2.0	0.03	99
100 nm	2.3	0.07	99

ZnO NPs	LC50 of NP _(total) (mg/L)	Toxic contribution (%)	
		NP _(ion)	NP _(particle)
Nanospheres	14.8	42	58
Nanosticks	8.9	34	66
Cuboidal SMPs	12.4	24	76

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Conclusions

Metal NP _(particle)	LC50s values (mg/L)
Cu	0.6
	1.7
	1.9
ZnO	20.3
	10.5
	12.1

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Relatieve bijdrage	Ionen (%)	Deeltjes (%)	
Cellijnen	20	80	Song, Vijver <i>et al.</i> 2014
Zebravis	30	70	Hua, Vijver <i>et al.</i> submitted

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Cladocerans – CuNP toxiciteit

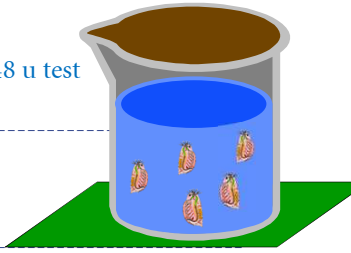


Experiment

Neonaten <24 uur, 48 u test
6 conc x 4 repl

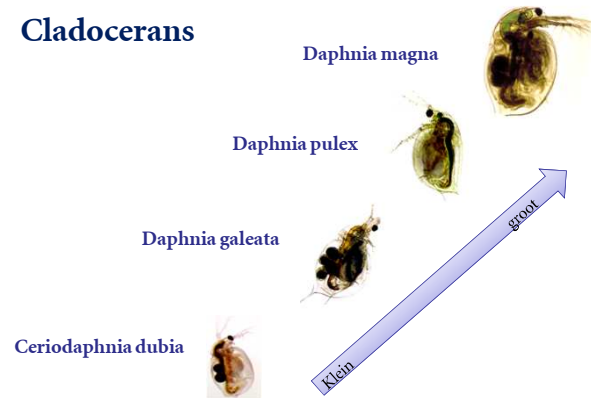
Mortaliteit
Grootte neonaten

Ionen & total metals
Aggregatie deeltjes
Stabiliteit



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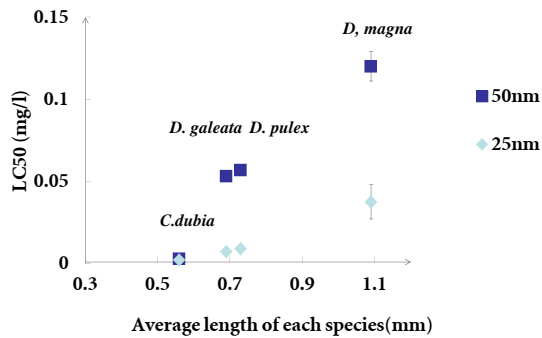
Cladocerans



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LC₅₀ CuNPs vs gemiddelde soort grootte

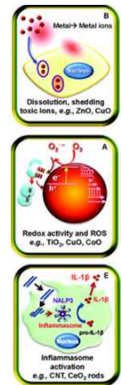
Song ... Vijver 201x



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Type response

- Dissolution of toxic ions
 - Metallothionein induction
- Oxidative stress
- Inflammation
- Trojan horse effects
- Behavioral effects
- Altered metabolism



Nel et al. (2013) Nanomaterial toxicity testing in the 21st century: Use of a predictive toxicological approach and high-throughput screening. Accounts for Chemical Research.

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Experimentele uitdagingen

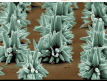
- Eendeloos veel deeltjes en grootten en vormen
- Erg veel blootstellingscondities
→ Beïnvloeden agglomeratie/ sedimentatie/ oplosbaarheid
= DYNAMISCH blootstelling
- Hoe onderscheid maken tussen:
 - Sorptie
 - Ingestie
 - Internalisatie
 Terwijl iedereen accumulatie + adsorptie rapporteert
- Onttrafelen relatieve contributie van deeltjes en ionen
- Biokinetische modellering
 - Opname en eliminatie fluxen

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Prospect

- Inzicht in mechanismen
- Chronic, foodweb effecten relevant
- Methodiek
- Verfijnde risico-inschatting:
 - Nieuwe nanodeeltjes
 - Ongeteste organismen



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Inbedding & samenwerking



VAST Bio Imaging +
robot voor ecotoxtesten

Cell Observatory
Universiteit Leiden



Synthese

Zelinsky Instituut
(Rusland)
Universiteit Leiden

REACH EU beleid

RIVM



COMPLIANCE

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Nano-ecotox researchers @ LU



- Development of an Adverse Outcome Pathway (AOP) for metallic nanoparticles
- Assessment of fate and effects of nanoparticles in aquatic systems
- Understanding the uptake and internal distribution of metallic nanoparticles in zebrafish embryos
- Effect of nanoparticles on microbial catabolism and community structure
- Development of quantitative nanostructure activity relationship (QSAR) models predicting the toxicity of metal-based nanoparticles to aquatic species
- LCA of Nanowire based Tandem Solar Cells

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